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Award Number: DAMD17-03-1-0037

TITLE: RAR Beta: Actions in Prostate Cancer

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REPORT DATE: April 2004

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
              Fort Detrick, Maryland 21702-5012

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20041118 043

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE April 2004	3. REPORT TYPE AND DATES COVERED Annual Summary (1 Apr 2003 - 31 Mar 2004)	
4. TITLE AND SUBTITLE RAR Beta: Actions in Prostate Cancer		**5. FUNDING NUMBERS** DAMD17-03-1-0037	
6. AUTHOR(S) Yong Zhuang, Ph.D.			
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Weill Medical College of Cornell University New York, New York 10021 *E-Mail:* yzhuang5@hotmail.com		**8. PERFORMING ORGANIZATION REPORT NUMBER**	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012		**10. SPONSORING / MONITORING AGENCY REPORT NUMBER**	

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited	12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 Words)

I have previously identified some RARβ targets genes. In the past year, I carried out actinomycin D experiments and determined that the RARβ targets genes are transcriptionally regulated by $RAR\beta_2$. In addition, I have shown that RARβ selective agonists and RXR agonists synergistically induce $RAR\beta_2$ target genes in F9 wild-type cells. I also showed that ELF3, one of RARβ target genes, was up-regulated by RA in normal human prostate epithelial cells, but not in human prostate tumor cells.

14. SUBJECT TERMS No Subject Terms Provided.			15. NUMBER OF PAGES 9
			16. PRICE CODE
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

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Introduction

Retinoids, a group of natural and synthetic analogues of vitamin A (retinol), can modulate cell growth and differentiation in vitro and in vivo (Gudas et al., 1994). An inverse relationship between retinoids levels and carcinogenesis has been observed over the last two decades. Vitamin A deficiency in experimental animals is associated with a higher incidence of cancer and with increased susceptibility to chemical carcinogens (Moon et al., 1994). Further, epidemiological studies showed that individuals with a lower dietary vitamin A intake are at a higher risk to develop cancer (Hong and Itri, 1994). Experimental models of carcinogenesis have demonstrated the efficacy of pharmacological levels of retinoids in preventing the development of cancers of the skin, oral cavity, lung, mammary gland, prostate, bladder, liver, and pancreas in animals exposed to carcinogenic agents (Moon et al., 1994). Thus, strong evidence exists that retinoids can prevent some types of cancers. Much in vitro and in vivo data indicate that retinoids play a role in the prevention of prostate cancer (Nanus and Gudas, 2000).

The biological effects of retinoids are mainly mediated by two classes of nuclear retinoid receptors: retinoic acid receptors (RARs) and retinoid X receptors (RXRs) (Mangelsdorf et al., 1994). The receptor RARβ is not expressed in a number of malignant tumors, including prostate cancer, lung carcinoma, squamous cell carcinoma of the head and neck, breast cancer, and esophageal carcinoma (Hu et al., 1991; Swisshelm et al., 1994; Zhang et al., 1994; Xu et al., 1994; Qiu et al., 1999). It has been suggested that the decrease in RARβ expression may lead to resistance to the growth inhibitory actions of retinoids. Indeed, transfection of RARβ into RARβ-negative cervical, breast, and lung cancer cells increased cell responsiveness to growth inhibition and induction of apoptosis by retinoids (Li et al., 1998; Si et al., 1996; Seewaldt et al., 1995; Liu et al., 1996; Weber et al., 1999). Stable expression of RARβ in a RARβ-negative prostate cancer cell line, PC-3, also potentiates the growth inhibitory effects of vitamin D3 analog and retinoids (Campbell et al., 1998).

In order to understand the function of the transcription factor RARβ, it is imperative that target genes regulated by RARβ are identified and analyzed. I have already successfully performed experiments to identify some of the target genes of RARβ in F9 cells by the use of subtractive hybridization and DNA expression micorarray techniques. By employing these two methods, I have identified many target genes, including transcription factors, protein tyrosine kinases, homeobox proteins, oncoproteins, ion channels, etc. Northern blot analysis was used to examine the regulation of the expression of these putative RARβ target genes by RA both in F9 Wt and F9 RARβ$_2$-/- cells.

Body

I carried out actinomycin D experiments to determine if the target genes I identified are transcriptionally regulated by RARβ. F9 wild type cells and F9 RARβ$_2^{-/-}$ cells were first treated for 12 h with or without 1 µM RA. At 12 h, RNA was isolated from some dishes of cells, while actinomycin D (2 µg/ml) was added to other dishes of cells to inhibit RNA transcription. RNA was isolated after an additional 6 h. The RNA samples were analyzed by Northern blot analysis (Figure 1). RA induced the target gene expression in F9 wild-type cells, but not in the F9 RARβ$_2^{-/-}$ cells. Treatment of wild type

cells with actinomycin D alone resulted in a decrease in target gene mRNA. Furthermore, RA induction of target gene mRNA was completely blocked by treatment with actinomycin D, indicating that ongoing transcription is required for RA-induced expression of target genes. For c-myc, the mRNA level seen after 18 h of RA treatment in F9 RAR$\beta_2^{-/-}$ cells was greatly reduced after 6 h of actinomycin D treatment, which is consistent with the previous finding that the half-life of c-myc transcripts in F9 cells is about 40 min.
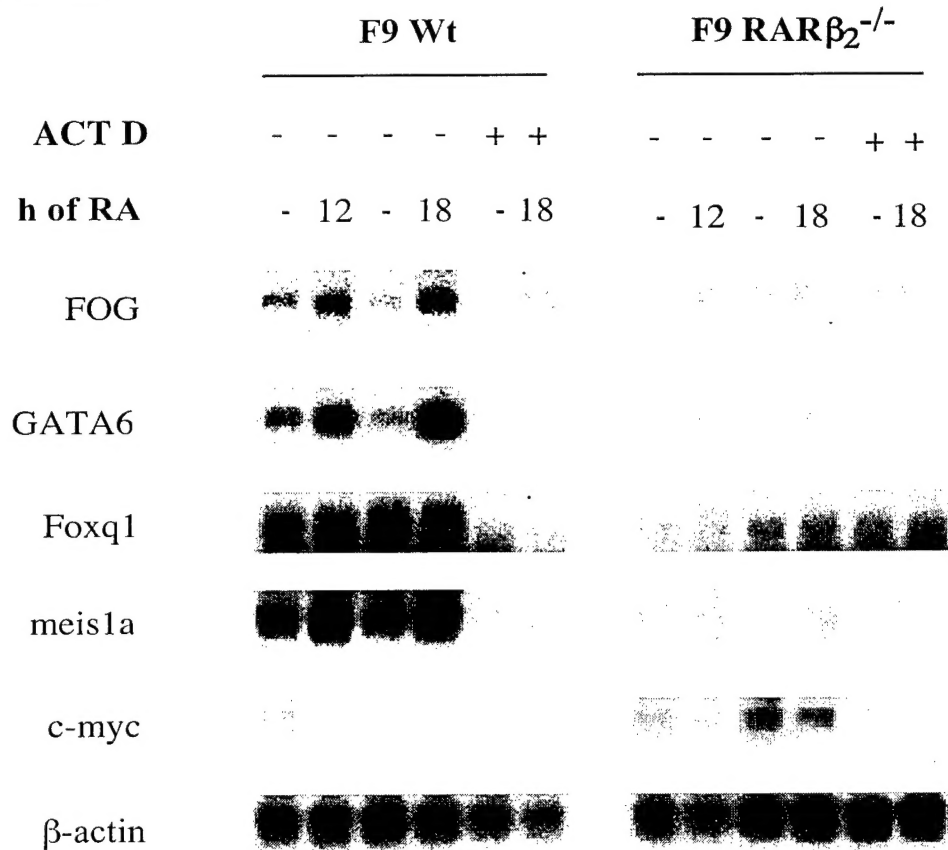
	F9 Wt						**F9 RAR$\beta_2^{-/-}$**					
ACT D	-	-	-	-	+	+	-	-	-	-	+	+
h of RA	-	12	-	18	-	18	-	12	-	18	-	18
FOG												
GATA6												
Foxq1												
meis1a												
c-myc												
β-actin												

Figure 1. Northern blot analysis of the effects of actinomycin D on RARβ_2 target gene expression. Wild-type F9 cells and F9 RAR$\beta_2^{-/-}$ cells were grown for 12 h in the absence (-) or presence (+) of 1 μM RA. At 12 h, RNA was isolated or 2 μg/ml transcriptional inhibitor actinomycin D (ACT D) was added to the medium and RNA was isolated after 6 h. After size-fractionation on the gel, the RNA was transferred onto nylon and hybridization to different cDNAs for the target genes was performed. This experiment was performed twice with similar results; one experiment is shown.

I also carried out agonist assays. F9 Wt and RAR$\beta_2^{-/-}$ cells were treated for 48 h with 1 μM RA or RARβ selective agonists (100 nM BMS185411 and 1 μM CD2314), and/or 1 μM pan-RXR agonist (BMS188,649 [BMS649]). BMS185411 or CD2314 alone didn't induce the expression of any target genes in F9 Wt cells. Similarly, BMS649 (pan-RXR) alone had no effect on the expression of target genes. However, BMS649 (pan-

RXR) and the RARβ selective agonists (BMS185411 or CD2314) synergistically induced the expression of all target genes in F9 Wt cells, but not in F9 RARβ$_2^{-/-}$ cells (Figure 2). Thus, RXR-RARβ$_2$ heterodimers appear to play a major role in mediating the induction of the expression of RARβ$_2$ target genes.
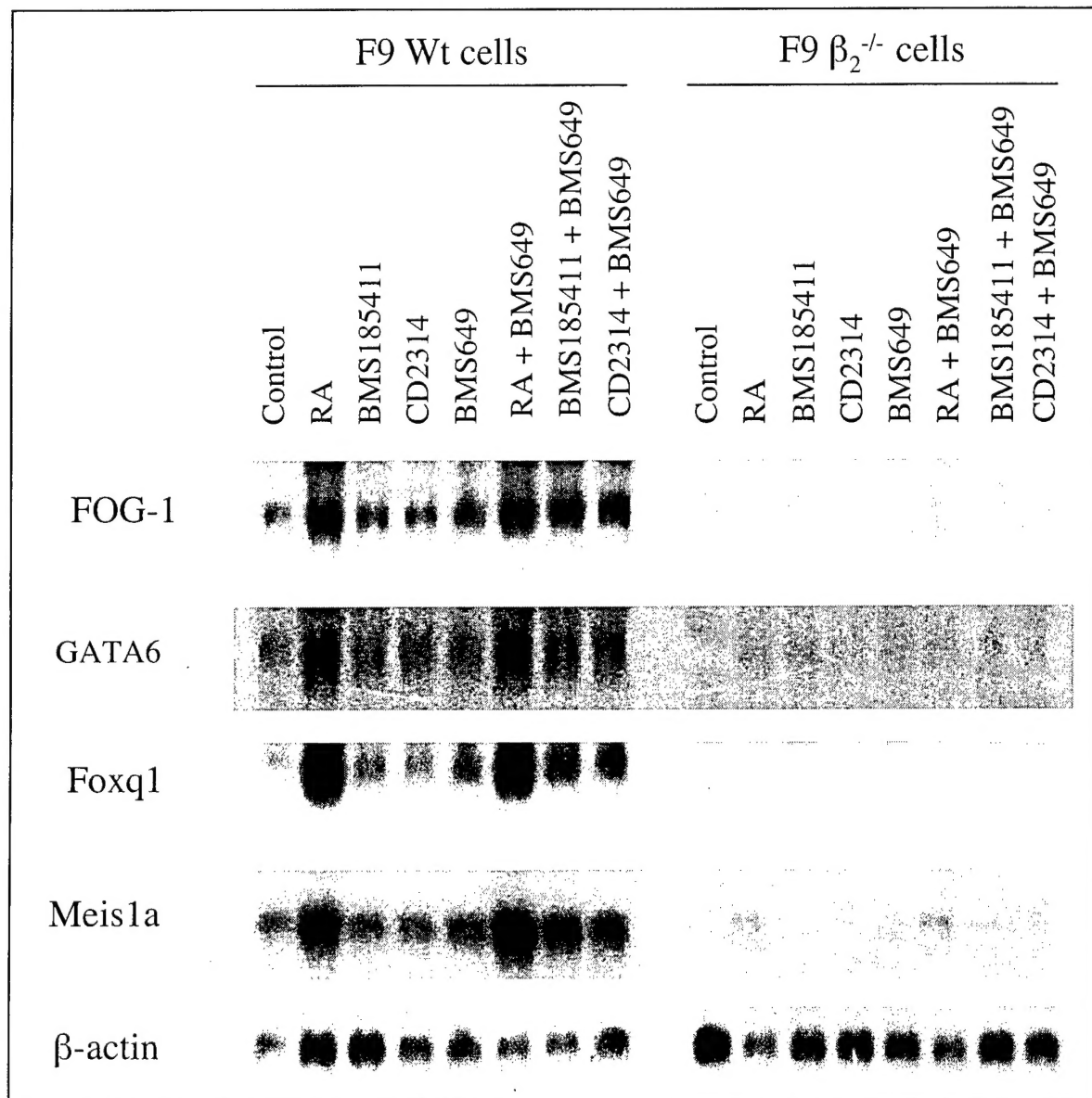

Figure 2. Expression of RARβ$_2$ target genes is increased by a RARβ-specific agonist and pan-RXR agonist. F9 wild-type cells and RARβ$_2^{-/-}$ cells were treated for 48 h with 1 μM RA, 1 μM or 100 nM RARβ-specific agonist (BMS185411 or CD2314), 1 μM pan-RXR agonist (BMS188649), or different combinations of drugs. Total RNA was then extracted and size-fractionated on the gel. The RNA was transferred onto nylon and hybridized to different cDNAs for the target genes. The experiments have been repeated two times with different RNA preparations. β-actin is used as an RNA loading control. One representative experiment is shown here.

ELF3 is a new RARβ target I have identified. ELF3 is a novel member of the Ets family of transcription factors. ELF3 is found to be specifically expressed in epithelial cells and in adult human tissues such as prostate, lung and kidney, which contain a significant proportion of epithelial cells (Tymns et al., 1997). ELF3 plays a role in epithelial differentiation and has been shown to suppress basal keratin 4 promoter activity in both esophageal and cervical epithelial cancer cell lines (Brembeck et al., 2000). Thus, ELF3 may play an important role in epithelial cancers of the prostate. I have also shown that the ELF3 mRNA level is lower in the lung and kidneys from RARβ knockout mice than from wild type mice.

I thus tested the expression of ELF3 mRNA in normal human prostate epithelial cell strains vs. prostate cancer cell lines. I carried out this study by using PrEc cells, a normal prostate epithelial cell line, and PC3 cells, a prostate cancer line. These cells were treated with 1μM RA for different times, mRNA was extracted, and Northern analysis was performed. ELF3 mRNA is not detectable in either control PrEc cells or PC3 cells. RA treatment for 24 h and 48 h resulted in a 5-fold induction of ELF3 mRNA in PrEc cells, but did not have an effect in PC3 cells (Figure 3). Since PC3 cells do not express RARβ, this result suggests that key RARβ target genes are not properly regulated in human prostate cancer as compared to normal cultured human prostate epithelial cells. I am currently examining expression of many other RARβ target genes in PrEc and PC3 cells.
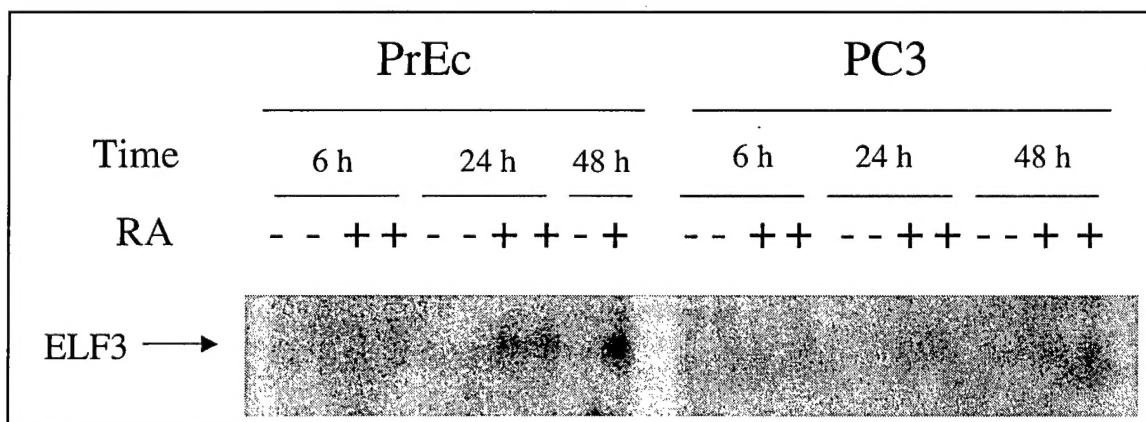

Figure 3. PrEC cells and PC3 cells were treated for 6 h, 24 h and 48 h with 1 μM RA, Total RNA was then extracted and size-fractionated on the gel. The RNA was transferred onto nylon and hybridized to cDNA for ELF3.

Key research Accomplishments

• I carried out Actinomycin D experiments to determine if the target genes are transcriptionally regulated by $RAR\beta_2$.

• I determined whether RARβ agonists and /or RXR agonists can stimulate the expression of the RARβ target genes.

• I studied the effects of retinoic acid on the expression of key RARβ target genes (eg. ELF3) in normal human prostate epithelial cells and prostate tumor cells.

Conclusions

I conclude that the RARβ targets genes are transcriptionally regulated by $RAR\beta_2$. In addition, I have shown that RARβ selective agonists and RXR agonists synergistically induce $RAR\beta_2$ target genes in F9 wild-type cells. I also showed that ELF3, one of RARβ target gene, was up-regulated by RA in normal human prostate epithelial cells, but not in human prostate tumor cells.

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